

Response of citrus hybrids to *Alternaria alternata* inoculation

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Abstract

Citrus orchards have some limitations, such as the occurrence of phytosanitary problems. *Alternaria* brown spot (ABS) is caused by fungus *Alternaria alternata*, which affects several parts of the plant by producing a host-specific toxin, known as ACT. ABS is a limiting factor in orchards due to the susceptibility of most planted cultivars: 'Murcott' tangor and 'Ponkan' tangerine. The selection of varieties resistant/tolerant to the disease has economic importance. Therefore, the aim of this experiment was to evaluate the response to *A. alternata* inoculation in a population of 'Murcott' tangor vs 'Pera' sweet orange hybrids. Leaves of 2-3 centimeters in length of 'Murcott' tangor, 'Pera' sweet orange, 'Ponkan', 'Dancy', 'Fremont' tangerine and 198 hybrids were collected. For *in vitro* inoculation, monospore *A. alternata* culture at concentration of 10^5 conidia mL⁻¹ was used. Inoculated leaves were stored in humid chamber. After 24, 48 and 72 hours of inoculation, leaf lesions were evaluated following a diagrammatic scale. The results obtained showed that most hybrids from the crossing of 'Murcott' tangor vs 'Pera' sweet orange are susceptible to ABS. However, 44 are resistant and ten are tolerant. Among ABS-tolerant hybrids, some have phenotype similar to that of cultivated and commercialized hybrids.

Keywords: *Citrus*, phytopathology, *alternaria* brown spot, mandarin, tangor

Introduction

The third predominant group in Brazilian citrus orchards is composed of tangerines and their hybrids, which are produced for the fresh fruit market (IBGE, 2018). Varieties that stand out are 'Ponkan' tangerine (*Citrus reticulata* Blanco) and 'Murcott' tangor hybrid (*Citrus reticulata* x *Citrus sinensis* (L.) Osbeck) (Bastianel et al., 2014).

Despite being competitive, Brazilian citrus has limitations, reflected in its planted area and production, which have suffered falls in recent years (Barros et al., 2016), which is the result of the reduced number of varieties and the monoculture system adopted, that is, by the narrow genetic base. These factors made the crop vulnerable to phytosanitary problems, which result in low productivity (Sousa & Goes, 2010; Wu et al., 2014). One of the main diseases that limits production and cultivation of tangerines is the *alternaria* brown spot (ABS).

ABS is caused by filamentous fungus *A. alternata* f. sp. *citri* (Azevedo et al., 2019). The disease was first observed in Brazil in 2001 in the 'Dancy' tangerine variety (*Citrus reticulata* Blanco) (Goes et al., 2001). Symptoms usually appear 24 to 48 hours after infection, affecting immature green tissues, causing defoliation and dryness in branches. In fruits, it promotes fall and the appearance of depressed lesions. Symptoms are characterized by black or brown spots, surrounded or not by yellow halos (Azevedo et al., 2015). Necrotic areas are the result of the action of the host-specific toxin, in the case of the tangerine pathotype, the toxin is ACT (*Alternaria Citri Tangerine*) (Tsuge et al., 2013). The fungus is saprophytic, surviving in plants remains and its reproduction is characterized by the production of conidia (Azevedo et al., 2010).

According to Bastianel et al. (2014), commercial varieties, such as 'Dancy' and 'Ponkan' tangerines

and 'Murcott' tangor are susceptible to ABS. Due to susceptibility, there is reduction in harvested area and increase in production costs, since orchards with susceptible varieties require more than 10 sprays per year to control the disease (Soriano et al., 2012). However, there are also resistant species, such as sweet oranges, 'Tahiti' acid lime (*Citrus latifolia* Tanaka), 'Montenegrina' tangerine (*Citrus delicious* Tenore) and 'Fremont' tangerine (*Citrus reticulata* Blanco x *Citrus clementina* Hort. Ex Tanaka) (Soriano et al., 2012; Bastianel et al., 2014).

Genetic improvement should be highlighted in the selection of resistant varieties, as it enables the introduction of superior genotypes, adapted to edaphoclimatic conditions and resistant to diseases. Hybridization is one of the most used tools in breeding programs (Spósito et al., 2003). Varieties that are not infected by the fungus can be used as source of resistance in crosses directed to obtain ABS-resistant hybrids.

It is understood that ABS represents losses for the sector, mainly due to the susceptibility of the main tangerine varieties. Thus, the aim of the present study was to evaluate the response of hybrids from the crossing of 'Murcott' tangor vs 'Pera' sweet orange (*Citrus sinensis*) to the *in vitro* inoculation of fungus *A. alternata* f. sp. *citri*, aiming to find tolerant or resistant varieties and attractive to the consumer market.

Material and Methods

Plant material

Hybrids were obtained in 1997 after controlled crosses between 'Murcott' tangor and 'Pera' sweet orange from the Citrus Germplasm Bank, at the "Sylvio Moreira" Citriculture Center of "Instituto Agrônômico" - IAC, located in Cordeirópolis-SP. Zygotic embryos were identified by using SSR markers (Oliveira et al., 2002) and grafted on 'Rangpur' lime (*Citrus limonia* Osbeck).

A total of 335 hybrids were obtained, which were established in 2015 in greenhouse; however, only 198 hybrids were included in the experiment. The two respective parents and three controls, 'Ponkan' and 'Dancy' tangerines, both susceptible to ABS and ABS-resistant 'Fremont' tangerine (Azevedo et al., 2010) were also evaluated in the presence of *A. alternata* isolate. The experiment was established at the "Sylvio Moreira" - IAC Citriculture Center.

Inoculation isolation and preparation

A. alternata isolate was obtained from 'Murcott' tangor fruit tissues with typical lesions, collected from plants maintained in field at the "Sylvio Moreira" - IAC

Citriculture Center, where the fungus occurs endemically. Injured tissues were cut, selected and superficially disinfected with immersion in 70% ethanol, 3% sodium hypochlorite and autoclaved distilled water.

Tissues were incubated in Petri dishes containing BDA culture medium (200 g potato, 20 g dextrose and 15 g agar L⁻¹) and carbendazin fungicide at concentration of 640 mg L⁻¹ of i.a. to inhibit the growth of opportunistic fungi and because *A. alternata* has high degradation capacity of this fungicide (Pacheco et al., 2012; Huang et al., 2015). Petri dishes were kept in BOD incubator with 12-hour photoperiod at 27°C. A monosporic culture was obtained from the initial isolate, according to adaptation of the methodology described by Silva et al. (2009).

After incubation period of approximately five days, the isolate was identified by means of asexual reproduction structures, using optical microscopy, observing dark colored conidia, with cross-sectional and longitudinal septa (Figure 1). The pathogenicity of the isolate was validated under laboratory conditions with *in vitro* inoculation tests on leaves of species known to respond to *A. alternata* such as 'Pera' sweet orange and 'Fremont' and 'Dancy' tangerines. After validating the pathogenicity, the experiment was continued with the other Petri dishes to prepare the inoculum solution.

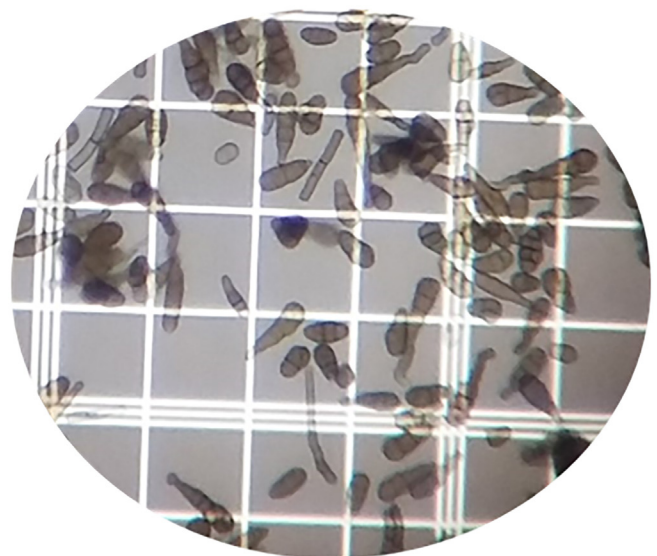


Figure 1. *A. alternata* f. sp. *citri* conidia and hyphae observed under microscope (40x).

The conidia suspension for leaf inoculation was obtained by adding 10 mL of autoclaved distilled water to the surfaces of Petri dishes. Conidia were removed with the aid of a sterile spatula, thus, obtaining the suspension, which was filtered with a double layer of sterile gauze to remove mycelial fragments. The final concentration was adjusted using hemocytometer (Neubauer chamber) for

10^5 conidia mL⁻¹.

In vitro Inoculation and evaluation of symptoms

For *in vitro* inoculations, leaves of branches in the V4 stage of vegetative development were used, standardizing with two to three centimeters in length. Petri dishes were lined with filter paper and a portion of cotton wool moistened with autoclaved distilled water to form a moist chamber and ensure favorable conditions for fungus infection. Leaves were packed with the abaxial side facing upwards (four leaves per plate) and 2.0 mL of conidia suspension were sprayed, according to methodology described by Peever et al. (1999), Canihos et al. (1999) and Turgutoğlu & Baktir (2019). Petri dishes were kept in BOD incubator chamber at temperature of 27°C and 12-hour photoperiod. Disease evaluations were performed 24, 48 and 72 hours after inoculation, using the diagrammatic scale described by Martelli et al. (2016). The scale represents the level of symptoms in ten scores, in which zero represents leaf without symptoms, and scores from one to nine represent, respectively, 0.3; 3.5; 8.0; 15.0; 34.0; 61.0; 80.0; 90.0 and 97.0% of leaf area with *A. alternata* symptoms.

The area under the disease progress curve (AUDPC) was calculated using the formula: $AUDPC = \sum [(y_1 + y_2) / 2 * (t_2 - t_1)]$, in which y = severity and t = time unit (Shaner & Finney, 1977). The experimental design was completely randomized, containing one treatment with three times (24, 48 and 72 hours), its control and four replicates. For statistical analysis, the SASM - Agri software (Canteri et al., 2001) was used. Data were transformed into square root ($Y + 1$) and tests of analysis of variance and comparison of means were performed using the Scott-Knott test at 5% probability level.

Results and discussion

Symptoms were observed 24 hours after fungus inoculation on leaves, such as small brown to black spots of varying size, characteristic ABS symptoms, which, in some cases, have expanded to large necrotic areas of leaf tissues due to the action of the specific-fungus toxin (ACT) (Akimitsu et al., 2014). In susceptible hybrids, such as TMxLP 241 and TMxLP 34, lesions gradually increased with time and the most severe lesions were found at time of 72 hours (Figure 2).

As expected, 'Ponkan' and 'Dancy' tangerines and 'Murcott' tangor presented lesions characteristic of alternaria brown spot, confirming their susceptibility (Bastianel et al., 2014) and differing statistically from 'Fremont' tangerine and 'Pera' sweet orange, used as controls (Porcino et al., 2017) resistant to the isolate that causes ABS (Figure 3). The resistance of 'Pera' sweet

orange is explained by the fact that the tangerine pathotype of *A. alternata* f. *citri* sp affect only tangerines and their respective hybrids, since it produces host-specific toxins (Akimitsu et al., 2014).

In the hybrid population, great variability was observed in response to *A. alternata* inoculation (Table 1).

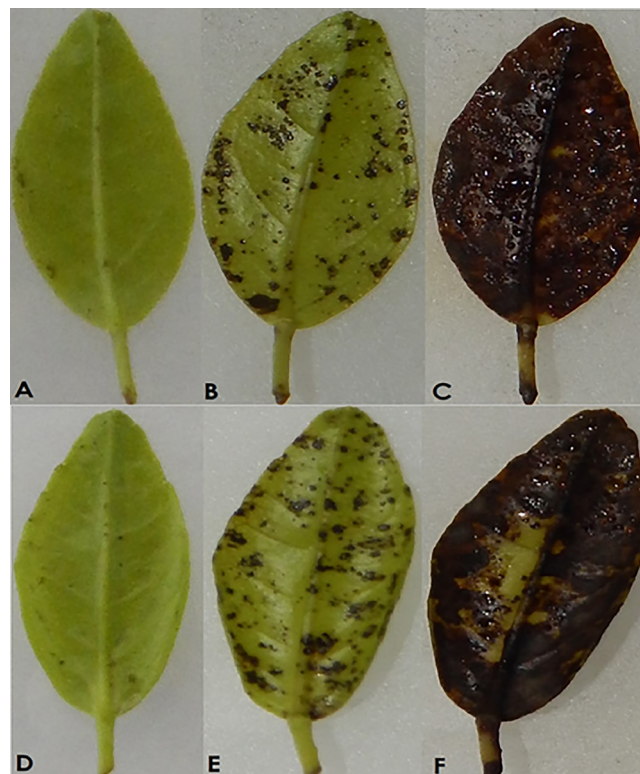


Figure 2. Evolution of alternaria brown spot lesions caused by *Alternaria alternata* in TMxLP 241 (A - 24 hours, B - 48 hours, C - 72 hours) and TMxLP 34 hybrids (D - 24 hours, E - 48 hours, F - 72 hours).

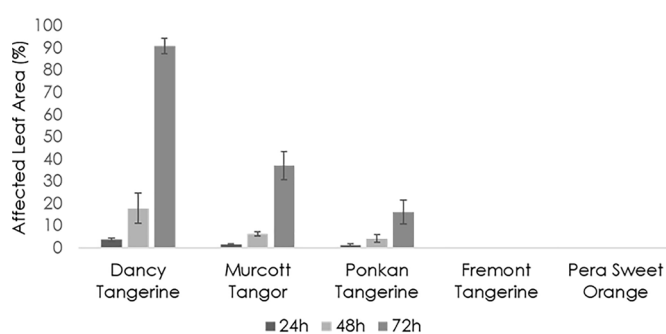


Figure 3. Percentage of leaf area affected by alternaria brown spot in varieties used as controls after 24, 48 and 72 hours of inoculation.

After 24 hours, 142 hybrids and three controls showed symptoms ranging from 0 to 15% of affected leaf area, corresponding to 71.43% of the population. In the 48-hour evaluation, eight more hybrids showed symptoms, ranging from 0 to 61% of affected leaf area, totaling 153 symptomatic individuals, corresponding

Table 1. Severity (%) after 24, 48 and 72 hours of inoculation and area under the disease progress curve (AUDPC), caused by *Alternaria alternata* in 'Murcott' tangor vs. 'Pera' sweet orange hybrids, their respective parents and controls, negative ('Fremont' tangerine) and positive ('Ponkan' tangerine) (Cordeirópolis, 2018).

| Individuals | Symptoms after inoculation (%) | | | | | AUDPC | | |
|-------------|--------------------------------|---|-------|---|-------|-------|---------|---|
| | 24h | | 48h | | 72h | | | |
| TMxLP 72 | 2.30 | d | 12.69 | c | 97.00 | a | 1496.16 | b |
| TMxLP 234 | 0.66 | e | 10.94 | d | 97.00 | a | 1434.48 | b |
| TMxLP 361 | 4.06 | c | 54.38 | a | 97.00 | a | 2517.84 | a |
| TMxLP 7 | 2.30 | d | 15.00 | c | 96.13 | a | 1541.16 | b |
| TMxLP 305 | 0.70 | e | 15.63 | c | 96.13 | a | 1537.08 | b |
| TMxLP 286 | 1.50 | d | 8.00 | d | 96.13 | a | 1363.56 | b |
| TMxLP 124 | 1.90 | d | 13.25 | c | 94.88 | a | 1479.36 | b |
| TMxLP 244 | 3.26 | c | 36.00 | a | 94.88 | a | 2041.68 | a |
| TMxLP 100 | 0.63 | e | 11.25 | d | 92.75 | a | 1390.56 | b |
| 'Dancy' | 3.70 | c | 17.90 | c | 91.00 | a | 1566.00 | b |
| TMxLP 208 | 0.70 | e | 44.63 | a | 90.38 | a | 2164.08 | a |
| TMxLP 225 | 8.06 | b | 44.12 | a | 90.12 | a | 2237.04 | a |
| TMxLP 203 | 3.10 | c | 14.13 | c | 87.38 | a | 1424.88 | b |
| TMxLP 192 | 2.30 | d | 11.41 | d | 86.75 | a | 1342.44 | b |
| TMxLP 61 | 0.86 | e | 5.63 | e | 86.73 | a | 1186.20 | b |
| TMxLP 80 | 2.82 | c | 27.88 | b | 83.25 | a | 1701.96 | a |
| TMxLP 342 | 0.19 | f | 12.13 | c | 82.88 | a | 1287.96 | b |
| TMxLP 215 | 4.06 | c | 13.25 | c | 82.63 | a | 1358.28 | b |
| TMxLP 220 | 3.10 | c | 42.56 | a | 82.00 | a | 2042.64 | a |
| TMxLP 347 | 2.70 | c | 8.38 | d | 81.63 | a | 1213.08 | b |
| TMxLP 44 | 2.70 | c | 16.88 | c | 80.50 | a | 1403.52 | b |
| TMxLP 71 | 4.06 | c | 13.06 | c | 78.38 | a | 1302.72 | b |
| TMxLP 136 | 3.50 | c | 10.38 | d | 77.13 | a | 1216.68 | b |
| TMxLP 64A | 3.26 | c | 14.50 | c | 76.25 | a | 1302.12 | b |
| TMxLP 149 | 0.26 | f | 21.75 | b | 75.87 | a | 1435.56 | b |
| TMxLP 24 | 0.19 | f | 8.79 | d | 74.41 | a | 1106.16 | b |
| TMxLP 232 | 0.26 | f | 21.25 | b | 74.38 | a | 1405.68 | b |
| TMxLP 336 | 1.50 | d | 10.06 | d | 72.88 | a | 1134.00 | b |
| TMxLP 74 | 0.23 | f | 13.31 | c | 71.75 | a | 1183.20 | b |
| TMxLP 118 | 3.10 | c | 10.38 | d | 71.13 | a | 1139.88 | b |
| TMxLP 325 | 3.50 | c | 23.14 | b | 69.25 | a | 1428.36 | b |
| TMxLP 304 | 1.06 | e | 21.31 | c | 68.87 | a | 1350.60 | b |
| TMxLP 273 | 1.90 | d | 13.56 | c | 68.63 | a | 1171.80 | b |
| TMxLP 69 | 5.57 | c | 48.37 | a | 68.44 | a | 2049.00 | a |
| TMxLP 277 | 0.19 | f | 4.23 | e | 67.75 | a | 916.80 | c |
| TMxLP 66 | 10.62 | a | 20.37 | b | 66.50 | a | 1414.32 | b |
| TMxLP 164 | 0.59 | e | 6.63 | e | 64.75 | a | 943.20 | c |
| TMxLP 205 | 0.66 | e | 10.94 | d | 64.63 | a | 1046.04 | b |
| TMxLP 57 | 0.19 | f | 8.14 | d | 63.50 | a | 959.64 | c |
| TMxLP 34 | 0.99 | e | 5.19 | e | 62.72 | a | 889.08 | c |
| TMxLP 343 | 2.57 | c | 30.07 | b | 61.10 | a | 1485.72 | b |
| TMxLP 228 | 1.46 | d | 26.57 | b | 60.54 | a | 1381.68 | b |
| TMxLP 284 | 2.86 | c | 16.56 | c | 60.12 | a | 1153.20 | b |
| TMxLP 108 | 0.15 | f | 7.10 | e | 59.38 | b | 884.76 | c |
| TMxLP 341 | 1.79 | d | 8.37 | d | 58.19 | b | 920.64 | c |
| TMxLP 46 | 2.66 | c | 14.12 | c | 58.00 | b | 1066.80 | b |
| TMxLP 240 | 0.30 | f | 8.06 | d | 57.88 | b | 891.60 | c |
| TMxLP 280 | 0.19 | f | 4.63 | e | 57.00 | b | 797.40 | c |
| TMxLP 248 | 1.06 | e | 9.50 | d | 56.88 | b | 923.28 | c |
| TMxLP 96 | 1.46 | d | 20.38 | b | 56.25 | b | 1181.64 | b |
| TMxLP 269 | 2.26 | d | 17.38 | c | 55.63 | b | 1111.80 | b |
| TMxLP 30 | 2.22 | d | 7.50 | d | 55.50 | b | 872.64 | c |
| TMxLP 379 | 0.90 | e | 9.40 | d | 55.50 | b | 902.40 | c |
| TMxLP 318 | 2.30 | d | 7.50 | d | 55.38 | b | 872.16 | c |
| TMxLP 47 | 0.70 | e | 6.23 | e | 55.19 | b | 820.20 | c |
| TMxLP 89 | 0.19 | f | 4.79 | e | 54.50 | b | 771.24 | c |

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|------------|------|---|-------|---|-------|---|---------|---|
| TMxLP 185 | 0.19 | f | 5.83 | e | 54.31 | b | 793.92 | c |
| TMxLP 311 | 1.46 | d | 12.44 | c | 54.30 | b | 967.68 | b |
| TMxLP 112 | 0.19 | f | 2.83 | e | 53.78 | b | 715.56 | c |
| TMxLP 306 | 0.63 | e | 5.66 | e | 53.25 | b | 782.40 | c |
| TMxLP 293 | 0.15 | f | 4.39 | e | 52.47 | b | 736.80 | c |
| TMxLP 365 | 0.23 | f | 4.23 | e | 52.38 | b | 732.84 | c |
| TMxLP 289 | 0.51 | e | 5.86 | e | 51.12 | b | 760.20 | c |
| TMxLP 350 | 1.10 | e | 5.19 | e | 50.88 | b | 748.32 | c |
| TMxLP 180 | 1.10 | e | 5.91 | e | 50.81 | b | 764.76 | c |
| TMxLP 186 | 0.35 | f | 2.04 | e | 49.75 | b | 650.16 | c |
| TMxLP 346 | 6.70 | b | 20.50 | b | 49.15 | b | 1162.20 | b |
| TMxLP 378 | 1.50 | d | 6.06 | e | 49.00 | b | 751.44 | c |
| TMxLP 287 | 0.11 | f | 7.74 | e | 47.20 | b | 753.48 | d |
| TMxLP 145 | 0.11 | f | 12.14 | c | 46.79 | b | 854.16 | c |
| TMxLP 337 | 1.46 | d | 6.06 | e | 46.62 | b | 722.40 | c |
| TMxLP 167 | 2.62 | d | 8.94 | d | 46.25 | b | 801.00 | c |
| TMxLP 101 | 1.86 | d | 6.97 | e | 45.06 | b | 730.32 | c |
| TMxLP 317 | 1.90 | d | 4.06 | e | 44.88 | b | 658.80 | c |
| TMxLP 39A | 0.22 | f | 8.79 | d | 42.45 | b | 723.00 | c |
| TMxLP 166 | 0.99 | e | 11.69 | d | 42.41 | b | 801.36 | c |
| TMxLP 363 | 1.42 | e | 3.02 | e | 41.69 | b | 589.80 | d |
| TMxLP 370 | 1.46 | d | 8.39 | d | 41.62 | b | 718.32 | c |
| TMxLP 230 | 2.30 | d | 9.81 | d | 41.37 | b | 759.48 | c |
| TMxLP 223 | 0.26 | f | 4.06 | e | 40.69 | c | 588.84 | d |
| TMxLP 241 | 0.59 | e | 6.14 | e | 40.60 | c | 641.64 | d |
| TMxLP 120 | 1.03 | e | 4.83 | e | 39.19 | c | 598.56 | d |
| TMxLP 235A | 0.55 | e | 6.70 | e | 38.62 | c | 630.84 | d |
| TMxLP 332 | 0.19 | f | 20.50 | c | 38.50 | c | 956.28 | c |
| TMxLP 49 | 2.86 | c | 9.25 | d | 38.37 | c | 716.76 | c |
| TMxLP 201 | 0.95 | e | 5.54 | e | 38.16 | c | 602.28 | d |
| TMxLP 373 | 0.91 | e | 6.14 | e | 38.12 | c | 615.72 | d |
| 'Murcott' | 1.46 | d | 6.30 | e | 37.04 | c | 613.20 | c |
| TMxLP 331 | 1.42 | e | 6.70 | e | 35.91 | c | 608.76 | d |
| TMxLP 219 | 0.15 | f | 5.66 | e | 35.81 | c | 567.36 | d |
| TMxLP 345 | 0.95 | f | 4.70 | e | 35.75 | c | 553.20 | e |
| TMxLP 177 | 0.19 | f | 8.06 | d | 35.00 | c | 615.72 | d |
| TMxLP 70 | 1.79 | d | 4.19 | e | 33.91 | c | 528.96 | d |
| TMxLP 99 | 0.11 | f | 4.84 | e | 33.81 | c | 523.20 | d |
| TMxLP 236 | 1.50 | d | 9.50 | d | 33.75 | c | 651.00 | c |
| TMxLP 321 | 0.62 | e | 2.86 | e | 33.72 | c | 480.72 | d |
| TMxLP 364 | 0.19 | f | 3.50 | e | 32.19 | c | 472.56 | d |
| TMxLP 250 | 0.59 | e | 5.30 | e | 31.97 | c | 517.92 | d |
| TMxLP 159 | 1.90 | d | 9.25 | d | 28.50 | c | 586.80 | d |
| TMxLP 307 | 0.23 | f | 9.19 | d | 28.25 | c | 562.32 | d |
| TMxLP 281 | 0.66 | e | 5.10 | e | 27.94 | c | 465.60 | d |
| TMxLP 187 | 0.55 | e | 3.30 | e | 27.56 | c | 416.52 | d |
| TMxLP 87 | 0.15 | f | 3.79 | e | 27.38 | c | 421.32 | d |
| TMxLP 295 | 0.15 | f | 8.22 | d | 26.81 | c | 520.80 | d |
| TMxLP 217 | 1.02 | e | 3.42 | e | 26.79 | c | 415.80 | d |
| TMxLP 90 | 0.04 | f | 6.88 | d | 25.63 | c | 473.16 | d |
| TMxLP 303 | 0.95 | e | 8.10 | d | 25.16 | c | 507.72 | d |
| TMxLP 268 | 0.51 | e | 1.50 | f | 23.75 | c | 327.12 | e |
| TMxLP 94 | 3.06 | d | 8.35 | e | 22.87 | d | 511.56 | d |
| TMxLP 247 | 0.95 | e | 2.39 | e | 22.85 | d | 342.96 | e |
| TMxLP 242 | 0.15 | f | 2.66 | e | 22.72 | d | 338.28 | e |
| TMxLP 278 | 0.51 | e | 3.42 | e | 22.70 | d | 360.60 | e |
| TMxLP 261 | 0.55 | e | 0.99 | f | 22.32 | d | 298.20 | e |
| TMxLP 182 | 0.66 | e | 4.06 | e | 19.94 | d | 344.64 | d |
| TMxLP 84 | 1.10 | e | 4.06 | e | 19.06 | d | 339.36 | e |
| TMxLP 152 | 0.15 | f | 6.66 | e | 18.97 | d | 389.28 | d |

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|-----------|------|---|-------|---|-------|---|--------|---|
| TMxLP 196 | 0.08 | f | 3.86 | e | 18.91 | d | 320.52 | e |
| TMxLP 338 | 0.00 | f | 6.06 | e | 18.87 | d | 371.88 | d |
| TMxLP 395 | 0.63 | e | 4.06 | e | 18.06 | d | 321.72 | e |
| TMxLP 42 | 0.19 | f | 1.90 | e | 17.41 | d | 256.80 | e |
| TMxLP 372 | 0.30 | f | 10.69 | d | 17.38 | d | 468.72 | d |
| TMxLP 176 | 0.08 | f | 1.64 | f | 16.91 | d | 243.24 | e |
| TMxLP 292 | 0.07 | f | 3.66 | e | 16.44 | d | 285.96 | e |
| 'Ponkan' | 1.42 | d | 4.26 | e | 16.17 | d | 313.32 | e |
| TMxLP 207 | 0.08 | f | 2.30 | e | 15.94 | d | 247.44 | e |
| TMxLP 195 | 1.02 | e | 2.70 | e | 15.82 | d | 266.88 | e |
| TMxLP 301 | 1.10 | e | 4.14 | e | 14.29 | d | 284.04 | e |
| TMxLP 227 | 0.15 | f | 7.97 | d | 13.31 | d | 352.80 | d |
| TMxLP 349 | 0.66 | e | 2.66 | e | 13.19 | d | 230.04 | e |
| TMxLP 111 | 0.08 | f | 4.14 | e | 12.75 | d | 253.32 | e |
| TMxLP 351 | 0.15 | f | 6.23 | e | 12.13 | d | 296.88 | e |
| TMxLP 376 | 0.15 | f | 4.94 | e | 11.88 | d | 262.92 | e |
| TMxLP 359 | 0.00 | f | 2.22 | e | 11.22 | d | 187.92 | e |
| TMxLP 334 | 0.07 | f | 1.42 | f | 10.92 | d | 165.96 | e |
| TMxLP 275 | 0.04 | f | 3.66 | e | 10.69 | d | 216.60 | e |
| TMxLP 360 | 0.11 | f | 2.30 | e | 9.98 | d | 176.28 | e |
| TMxLP 116 | 0.48 | e | 0.99 | f | 9.69 | d | 145.80 | e |
| TMxLP 233 | 0.07 | f | 1.10 | f | 9.65 | d | 143.04 | e |
| TMxLP 246 | 0.00 | f | 0.19 | f | 9.61 | d | 119.88 | e |
| TMxLP 315 | 0.59 | e | 1.46 | f | 9.10 | d | 151.32 | e |
| TMxLP 55 | 0.04 | f | 2.23 | e | 8.85 | d | 160.20 | e |
| TMxLP 59 | 0.00 | f | 0.00 | f | 7.63 | d | 91.56 | f |
| TMxLP 60 | 0.00 | f | 4.79 | e | 7.50 | d | 204.96 | e |
| TMxLP 104 | 0.04 | f | 0.23 | f | 6.79 | d | 87.48 | e |
| TMxLP 4 | 1.03 | e | 3.26 | e | 5.50 | d | 156.60 | e |
| TMxLP 291 | 0.15 | f | 1.46 | f | 5.34 | d | 100.92 | e |
| TMxLP 328 | 0.04 | f | 0.99 | f | 5.34 | d | 88.32 | e |
| TMxLP 160 | 0.19 | f | 0.66 | f | 4.23 | e | 68.88 | e |
| TMxLP 200 | 0.00 | f | 1.50 | f | 3.26 | e | 75.12 | e |
| TMxLP 323 | 0.04 | f | 0.23 | f | 3.10 | e | 43.20 | f |
| TMxLP 324 | 0.00 | f | 0.00 | f | 2.04 | e | 24.48 | f |
| TMxLP 260 | 0.00 | f | 0.11 | f | 1.55 | e | 21.24 | f |
| TMxLP 97 | 0.00 | f | 0.95 | f | 1.50 | e | 40.80 | f |
| TMxLP 9 | 0.00 | f | 0.00 | f | 1.23 | e | 14.76 | f |
| TMxLP 221 | 0.08 | f | 0.08 | f | 1.08 | e | 15.84 | f |
| TMxLP 319 | 0.00 | f | 0.00 | f | 0.19 | e | 2.28 | f |
| TMxLP 11 | 0.00 | f | 0.04 | f | 0.07 | e | 1.80 | f |
| TMxLP 5 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 14 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 16 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 18 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 19 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 31 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 40 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 41 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 56 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 75 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 88 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 92 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 93 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 107 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 109 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 119 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 146 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 156 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 163 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 169 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |

Continue...

| | | | | | | | | |
|-----------|------|---|------|---|------|---|------|---|
| TMxLP 170 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 199 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 202 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 206 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 212 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 213 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 214 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 235 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 245 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 279 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 285 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 288 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 290 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 296 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 299 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 309 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 314 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 327 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 348 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 354 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 362 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 433 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 469 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| TMxLP 887 | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| 'Fremont' | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| 'Pera' | 0.00 | f | 0.00 | f | 0.00 | e | 0.00 | f |
| CV | 20% | | 30% | | 31% | | 33% | |

Averages followed by the same letter do not differ according to the Scott-Knott test at 5% probability.

to 75.37%. In the last evaluation at 72 hours, 154 hybrids showed symptoms ranging from 0 to 97% of affected leaf area, corresponding to 75.86% of the population evaluated in the experiment.

In total, 44 resistant hybrids were observed. Another ten hybrids were tolerant to the disease, since they had low levels of symptoms when compared to other hybrids and did not show statistical differences in relation to asymptomatic plants. It was observed that 86 hybrids presented severity greater than that presented by their susceptible parent, 'Murcott' tangor and nine individuals had severity greater than that of 'Dancy' tangerine, one of the varieties most susceptible to the disease, thus, these materials would not be recommended for areas with high incidence of the disease (Michielin et al., 2016).

TMxLP 281 hybrid, known as the recently launched 'IAC 2019 Maria' tangerine cultivar, stands out. In evaluations under field conditions, it was resistant to ABS; however, it presented mild symptoms (Table 1) in *in vitro* experiments. The fact that the inoculation was carried out on detached leaves explains in part this phenomenon, since they present physiological changes reflecting on their resistance level; detached leaves lose their power to respond to infections by pathogens (Azevedo et al., 2010; Bastianel et al., 2014).

Studies on the response to inoculation *in vivo*

hybrids are necessary to complement the results, aiming to evaluate the plant's response power to infections, since detached leaves do not have all mechanisms used in plant defense.

The materials used in this study are part of the breeding program of the "Sylvio Moreira" - IAC Citriculture Center. Some hybrids have been evaluated according to their physicochemical characteristics in previous studies, such as that by Michielin et al. (2016) and Curtolo et al. (2017). It was observed that part of the population of hybrids resembled tangors, tangerines and sweet oranges when visual characteristics such as fruit size, ease of peeling, peel thickness, number of segments and seeds were evaluated (Curtolo et al., 2017). Evaluations of soluble solids, ratio and productivity performed by Michielin et al. (2016) complemented the study, and it was possible to verify that some hybrids had agronomic characteristics similar to commercial varieties.

Among the 52 hybrids selected and evaluated by Michielin et al. (2016) 33 of them were also evaluated in the present work, such as TMxLP 14, 16, 47, 107, 111, 112, 116, 163, 180, 233, 279, 285, 324, 345, 354 hybrids, which resembled sweet oranges and TMxLP 9, 60, 61, 66, 71, 84, 99, 118, 124, 201, 281, 315, 317, 321, 336, 343, 370, 373 hybrids, which showed greater similarity to tangors.

There was greater susceptibility in the group of

tangors when compared to the group of sweet oranges, which presented seven hybrids resistant to ABS (Figure 4). Among hybrids of the tangor group, TMxLP 9, 60, 315 and 84 showed greater tolerance when compared with the others in their group, with emphasis on TMxLP 9, which did not differ statistically from asymptomatic hybrids, presenting potential for being tolerant to ABS, with phenotype similar to that of 'Murcott' tangor, currently one of the most cultivated and commercialized species. Tangor fruits are highly appreciated by the Brazilian consumer and excellent option for the domestic and foreign fresh fruit market (Michielin et al., 2016).

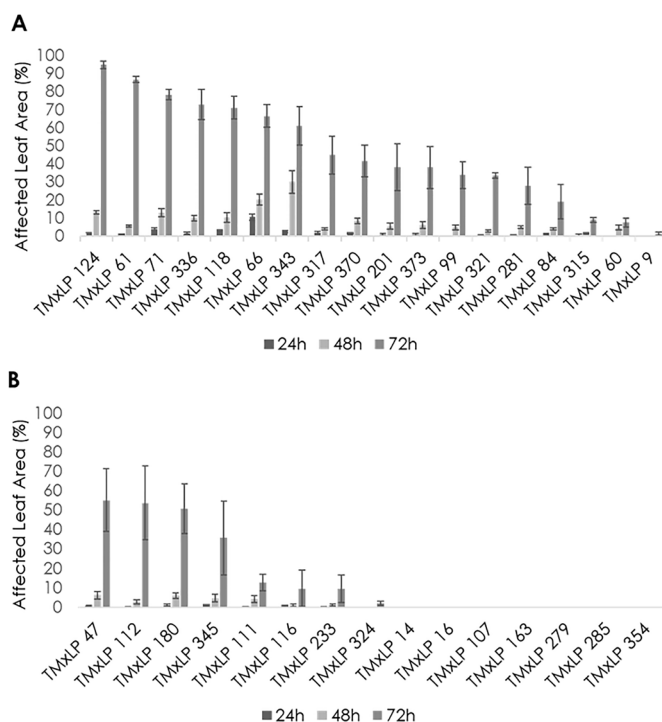


Figure 4. Percentage of leaf area affected by alternaria brown spot after 24, 48 and 72 hours of inoculation in citrus hybrids selected by the Breeding Program of the "Sylvio Moreira / IAC Citriculture Center. A - Group of tangors, B - Group of sweet oranges.

Although 52 hybrids were previously evaluated (Michielin et al., 2016), the present study represents an increase in the volume of collected data, since 198 hybrids were evaluated, supporting future studies on mapping and genomic selection, for example.

Regarding the 198 F1 progenies, it could be concluded that the proportion of susceptible of resistant was 154:44, which apparently corresponds to a 3:1 ratio. The segregation found in this study opposes the hypothesis that the inheritance of ABS resistance is controlled by a single recessive allele, advocated by authors such as Dalkilic et al. (2005); Gulsen et al. (2010); Cuenca et al. (2013) and Michielin et al. (2016).

The origin of the progeny can explain the divergence among segregations found in previous works, since different populations result in different segregations. The hybrids used in this study were obtained through controlled crosses between 'Murcott' Tangor and 'Pera' sweet orange. 'Murcott' tangor is widely known as a natural hybrid of sweet orange and tangerine. Therefore, it can be considered as a backcross, similar to progeny studied by Campos et al. (2017).

However, only population phenotypic data for ABS resistance do not allow identifying the dynamics of alleles involved in the trait. Therefore, genetic and molecular studies are necessary to explain the resistance or susceptibility to *A. alternata* in citrus.

Conclusions

Among the 198 hybrids evaluated, 44 are resistant and ten tolerant to *A. alternata*, demonstrating the greater susceptibility of the population in *in vitro* assays.

Among the 33 hybrids used in the experiment and which had already been evaluated by the breeding program of the "Sylvio Moreira" Citriculture Center, seven in the group of sweet oranges showed resistance and four in the group of tangors showed tolerance to ABS.

Tolerant hybrids, belonging to the group of tangors, have potential for being used in regions with high incidence of the disease, being an alternative to susceptible varieties.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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